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The Oral Pancreatic Function Test with N-Benzoyl-*L*-tyrosyl-*p*-aminobenzoic Acid: Acute Toxicity and Effects of Renal Function on this Test

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Summary: The oral pancreatic function test with N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid was performed on 24 healthy test subjects, and its toxicity was examined. Eight patients with restricted renal function and known renal disease were also investigated. The pancreatic function test and the same procedure using free *p*-amino-benzoic acid were performed at 2–3-day intervals.

During all the investigations with the pancreatic function test, no clinical side effects were observed. All parameters investigated at all the test times fell within the normal range. No toxicity of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid could be found. The excretion of *p*-aminobenzoic acid after administration of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid was greatly reduced in all patients with restricted renal function. Four of eight patients also showed essentially no increase in excretion rate when free *p*-aminobenzoic acid was given instead of the peptide. It is therefore not possible to correct the pancreatic function test results in patients with renal insufficiency by calculating the ratio of *p*-aminobenzoic acid excretion after peptide intake to that after free *p*-aminobenzoic acid ingestion. Adequate renal function is therefore a prerequisite for the pancreatic function test.

Der orale Pankreas-Funktions-Test mittels N-Benzoyl-L-tyrosyl-p-aminobenzoesäure: Akute Toxizität und Beeinflussung durch die Nierenfunktion

Zusammenfassung: Der orale Pankreas-Funktions-Test mittels N-Benzoyl-*L*-tyrosyl-*p*-aminobenzoesäure wurde an 24 gesunden Testpersonen durchgeführt und es wurde dabei nach toxischen Effekten gesucht. Acht Patienten mit eingeschränkter Nierenfunktion wurde außer dem Testsubstrat auch freie *p*-Aminobenzoesäure unter gleichen Bedingungen in 2–3-tägigem Abstand verabreicht.

Während der ganzen Prüfperiode mit dem Pankreas-Funktions-Test wurden keine klinischen Nebenwirkungen beobachtet. Alle zu den verschiedenen Testzeiten gemessenen Größen fielen in den Normalbereich und keine toxischen Effekte konnten festgestellt werden. Die Ausscheidung an *p*-Aminobenzoesäure war deutlich geringer bei allen Patienten mit Niereninsuffizienz. Vier dieser acht Patienten zeigten nach Verabreichung von freier *p*-Aminobenzoesäure keine wesentlich höhere Ausscheidung von *p*-Aminobenzoesäure als nach Einnahme des Peptids. Somit ist es nicht möglich, die Pankreas-Funktions-Test-Resultate von niereninsuffizienten Patienten mit Hilfe einer solchen Relation zu korrigieren. Adäquate Nierenfunktion ist also eine notwendige Voraussetzung für einen aussagekräftigen Pankreas-Funktions-Test.

Introduction

In 1972 *Imondi* et al. (1) reported successful experiments in animals with a new oral function test. The results were later confirmed in monkeys with metabolically induced pancreatic insufficiency (2). The test is based on the chymotrypsin-specific cleavage of the orally administered peptide, N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid. The *p*-aminobenzoic acid liberated is absorbed, partially metabolized in the liver, and excreted in the urine. The amount of *p*-aminobenzoic acid eliminated in the urine during a given period serves as a measure of exocrine pancreatic secretion. The pancreatic function test has also been applied to humans since that time (3), and on the basis of available reports it seems capable of fulfilling the expectations suggested by a screening procedure (4–8). The test is generally well tolerated. Till now no toxic side effects have been

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found (7). However, a systematic toxicity study has not previously been reported in detail (3–8).

Since *p*-aminohippuric acid, a metabolite of *p*-aminobenzoic acid, is used to test renal clearance, it would be expected that the excretion of *p*-aminobenzoic acid would be reduced after taking this peptide in the case of renal disease. There are no test results till now, which would permit evaluation of the pancreatic function test in relation to renal function.

The goal of the present investigation was to perform a systematic acute toxicity test in humans, and also to investigate the effect of renal function on the pancreatic function test.

Materials and Methods

Investigation of the acute toxicity of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid

Test subjects

Twenty-four healthy volunteers subjected themselves to the *Lundh* test and the oral pancreatic function test with N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid, and to the toxicity test. None of the individuals had pancreatic disease. They denied excessive alcohol intake. Age and sex are presented in table 1.

Tab. 1. Acute toxicity of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid: Basic data of persons tested.

No.	Sex	Age (a)	*Lundh* test[1]) (Trypsin, μmol $H^+/l \cdot s$)	Pancreatic function test[2]) (*p*-aminobenzoic acid excretion as % of the applied dose)
1	♂	26	145.8	67.5
2	♂	26	295.8	66.2
3	♂	27	583.3	67.5
4	♂	27	258.3	69.8
5	♂	26	200.0	76.8
6	♂	27	–	61.1
7	♂	25	375.0	70.0
8	♂	23	195.8	61.2
9	♂	26	58.3	62.5
10	♂	26	366.7	65.4
11	♀	25	495.8	77.5
12	♂	26	179.2	49.2
13	♂	24	325.0	62.0
14	♂	26	287.5	64.0
15	♂	27	275.0	62.3
16	♂	28	–	63.4
17	♂	26	345.8	62.1
18	♂	32	254.2	72.7
19	♂	38	366.7	52.1
20	♂	25	195.8	72.7
21	♀	25	187.5	65.2
22	♀	18	325.0	47.0
23	♀	21	141.7	67.0
24	♂	22	–	58.4

[1]) normal > 147 μmol $H^+/l \cdot s$, border-line 100–147 μmol $H^+/l \cdot s$, abnormal < 100 μmol $H^+/l \cdot s$.
[2]) normal ⩾ 47%.

Test procedure

The *Lundh* test was first carried out in the test subjects for the sake of comparison and to confirm intact pancreatic function. About 1 week later the pancreatic function test was performed, with 4 blood sample collections and urine examinations. The first blood sample was taken with the patient fasting prior to ingestion of 1 g of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid sodium salt and the other 3 at 6 hours, 24 hours, and 48 hours after ingestion of the peptide. Each blood sample was examined for the following parameters: hemoglobin, erythrocyte count, leukocyte count, differential white blood cell count; bilirubin, alkaline phosphatase, serum glutamate-oxalacetate transaminase, serum glutamate-pyruvate transaminase, prothrombin; urea, creatinine, sodium, potassium, calcium; serum amylase and blood sugar. A urine sample was also examined with the first and second blood samples. The urine samples were examined for pH, protein, glucose, ketone bodies, urobilinogen, urine lactate dehydrogenase, and sediment.

In six subjects ("controls") recruited from among the test subjects (table 1: Nos 1, 2, 11, 15, 16, 24), blood was taken in the fasting state and again 6 hours later. Urine was examined under the test conditions (fasting, equal liquid intake, avoidance of all drugs), but without administration of the peptide, in accordance with the conditions for the toxicity test. This was done to eliminate any artefacts in the toxicity test due solely to water intake.

Effect of renal function on the pancreatic function test

Patients

Examinations were performed on eight hospitalized patients in the Nephrology Department of the Kantonsspital Basel. These patients had reduced function with known chronic renal disease. The patients were told in detail about the study by their physicians and signed an "informed consent" form. Table 2 provides information on the age, sex, diagnosis, and renal function in these patients.

Control group

The *Lundh* test and the pancreatic function test results of 13 patients with demonstrably intact renal function were used as controls. The age, sex, diagnoses, and results of the *Lundh* test and the pancreatic function test are presented in table 3.

Test procedure

At intervals of at least 48 hours, but not more than 72 hours, the pancreatic function test and the same procedure with free *p*-aminobenzoic acid were carried out on the same patient. The *Lundh* test was performed at intervals of no more than 2 weeks before or after the two tests. Between the pancreatic function test and the intake of free *p*-amino-benzoic acid the serum creatinine and the creatinine clearance were determined. However, it proved impossible to perform the *Lundh* test in three of the patients because of their age, their serious primary illness, or a lack of cooperation.

Methods

Pancreatic function test

The pancreatic function test was performed as previously described (7).

Examination with free p-aminobenzoic acid

The patients with confirmed renal insufficiency were given 320 mg of *p*-aminobenzoic acid orally with 150 ml of water. The collection of control and 6-hour urine took place in the same manner as with the pancreatic function test.

Lundh test

The procedure of the *Lundh* test was described earlier (9–11). Mean trypsin activities of 100 μmol $H^+/l \cdot s$ are considered abnormal while those of 100–147 μmol $H^+/l \cdot s$ are regarded as borderline values (12).

Tab. 2. Results of the several tests in patients with renal insufficiency.

Name	Sex	Age (a)	Creatinine clearance (μl/s)	Serum creatinine[1]) (μmol/l)	Free *p*-amino-benzoic acid	Pancreatic function test[2])	*Lundh* test[3]) (Trypsin, μmol H^+/l·s)	Diagnosis
					(*p*-aminobenzoic acid excretion as % of the applied dose)			
G. M.	♀	60	167	362.4	15.8	14.0	295.8	Interstitial bacterial nephritis
Sch. A.	♂	45	300	459.7	27.0	25.8	175.0	Chronic sclerosing intracapillary glomerulonephritis
Z. L.	♂	39	1117	114.9	44.8	66.8	–	Acute exudative glomerulonephritis
W. E.	♂	55	183	371.3	27.7	22.1	204.2	Chronic interstitial nephritis
St. M.	♂	59	350	176.8	18.0	18.8	145.8	Interstitial toxic nephritis
P. L.	♂	79	383	194.5	18.8	8.2	241.7	Chronic interstitial nephritis
S. T.	♂	62	245	194.5	21.7	12.9	–	Interstitial toxic nephritis
J. M.	♀	86	217	150.3	52.5	37.3	–	Chronic interstitial nephritis

[1]) normal ≤ 105 μmol/l
[2]) normal ≥ 47%
[3]) normal > 147 μmol H^+/l·s, borderline 100–147 μmol H^+/l·s, abnormal < 100 μmol H^+/l·s

Tab. 3. Results of the oral pancreatic function test and the *Lundh* test in control patients with normal renal function.

Name	Sex	Age (a)	Serum creatinine[1]) (μmol/l)	Pancreatic function test (*p*-aminobenzoic acid excretion as % of the applied dose)	*Lundh* test[3]) (Trypsin, μmol H^+/l·s)	Diagnosis
Sch. J.	♂	74	79.6	62.6	383.3	Bronchial carcinoma
S. J.	♂	71	53.0	73.0	179.2	Tuberculosis
B. E.	♂	50	79.6	54.3	366.7	Irritable colon
F. K..	♀	75	97.2	58.6	295.8	Nontropical sprue
L. T.	♀	57	70.7	68.6	295.0	Duodenal ulcer
N. A.	♂	57	79.6	71.9	313.3	Dumping syndrome
U. F.	♂	70	106.1	57.2	308.3	Hyperlipemia
B. J.	♂	29	97.2	57.0	258.3	Gastric ulcer
W. S.	♂	31	61.9	49.4	412.5	Trauma of pancreas
H. B.	♀	69	53.0	78.6	154.2	Cholelithiasis
L. H.	♀	51	79.6	56.2	191.7	Hyperthyreodism
G. H.	♂	52	53.0	62.4	195.8	Peritoneal carcinosis
K. H.	♂	68	70.7	61.0	208.3	Choledochal stenosis

[1]) normal ≤ 105 μmol/l
[2]) normal ≥ 47%
[3]) normal > 147 μmol H^+/l·s, borderline 100–147 μmol H^+/l·s, abnormal < 100 μmol H^+/l·s

Blood chemistry studies

The biochemical investigations in the blood were performed on a Technicon SMA Multi-Channel-Analyzer (Technicon, Tarrytown, New York) in the Chemical Laboratory of the Kantonsspital Basel, except for serum glucose (13), serum amylase (14), and urine lactate dehydrogenase (15).

Statistics

The statistical evaluation of the results was carried out with the aid of *Student*'s t-test and regression analysis according to methods described by *Remington & Shork* (16).

Results

Acute toxicity of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid

Clinical aspects

The results of the pancreatic function test and the *Lundh* test are shown in table 1. The *Lundh* test could not be performed in three of the test subjects for technical reasons. The interval between the *Lundh* test and the pancreatic function test was greater than 4 weeks in three test subjects. One *Lundh* rest result was below the lower normal limit (58.3 μmol H^+/l · s). The pancreatic function test revealed an excretion of *p*-aminobenzoic acid within the normal range in this subject. The laboratory, clinical, and case history findings gave no indication of pancreatic disease. The test subjects were well at the time of the *Lundh* test, pancreatic function test and the venipuncture.

Blood and urine tests in the pancreatic function test

For technical reasons it was impossible to evaluate the prothrombin time in more than nine subjects. One subject was suspected of having *Meulengracht*'s disease because of increase in bilirubin with normal alkaline phosphatase and serum transaminases (table 1, No 20). The bilirubin value of this subject was not used.

None of the changes of the observed serum parameters went beyond the normal range. In the case of the serum parameters in which significant changes could be seen between the individual venipunctures, the serum values of the "controls" were also examined for such differences, which, except in the case of the transaminases, were found.

The results of the urine studies showed no indications of pathological changes. The sediment findings in particular were always stable. One individual displayed a urine lactate dehydrogenase value in excess of 60 IU/l in the first control (6-hour portion). The values during the subsequent investigations were in the normal range.

Effect of the renal function on the pancreatic function test

The results of the investigations with free *p*-aminobenzoic acid, N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid, and the results of the *Lundh* test, the diagnoses and values of serum creatinine and creatinine clearance of the eight patients are summarized in table 2. The excretion of *p*-aminobenzoic acid after ingestion of the peptide was markedly reduced in all patients with severely restricted renal function and correspondingly increased serum creatinine value. The pancreatic function test was in the normal range in all control patients with creatinine values between 53.0 and 106.1 μmol/l (tab. 3).

A highly significant correlation existed between the serum creatinine value and the excretion of *p*-aminobenzoic acid after ingestion of the peptide, if renal insufficiency patients and control patients were considered together ($r = 0.81$; $p < 0.001$). No definite correlation could be seen in the latter group alone.

In four of the eight patients with renal insufficiency the excretion rate of *p*-aminobenzoic acid was not clearly different whether ingested in the free or conjugated form (tab. 2). Similar results were obtained by *Bornschein* et al. (8) in subjects with normal renal function.

Discussion

Investigation of the toxicity of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid

In order to study possible acute toxic effects of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid, serum and urine parameters in healthy young test subjects were checked for significant changes. On the whole these parameters fell within the normal range, even though they varied within this range. All of the serum parameters, in which significant changes occurred between the individual venipunctures, were compared with the serum parameters (first and second venipunctures) of the "controls". This comparison revealed that the increasing and decreasing trends of the laboratory values in the two groups compared were always parallel. Therefore it can be assumed that the changes after 6 hours are mostly due to liquid intake alone. Only the transaminase values displayed a contrary trend. Between the first and second venipunctures the serum glutamate-oxalacetate transaminase decreased significantly in the test subjects, while it did not change in the "controls". However, one should keep in mind that these changes vary within the normal range. The question of the extent to which daily fluctuations in fasting individuals come into play must remain unanswered, since reports in this connection are available only with respect to longer periods of abstinence of nutrients (17).

Clinical side effects were never observed during the studies with pancreatic function test. No symptoms related to the gastrointestinal tract or central nervous system were reported.

Toxic effects of *p*-aminobenzoic acid and its metabolites are rarely mentioned in the literature, but have been described for doses several times higher than those used

in the pancreatic function test. *p*-Aminobenzoic acid has been used in rickettsiosis (1–4 g/24 hours) (18) and lupus erythematosus. At dosages which produced blood concentrations up to a maximum of 600–700 mg/l, nausea, vomiting, pruritus, and possible hepatitis were observed. In addition *p*-aminobenzoic acid caused hypoglycemic effects in patients on restricted diet, and was followed by leukopenia and agranulocytosis in some cases (18). Since only an equivalent of 320 mg of *p*-aminobenzoic acid is administered in the pancreatic function test, such side effects would scarcely be anticipated. The leukocyte count never changed significantly during the course of the investigation.

These data confirm the previous observations of our and *Bornschein*'s group (7, 8) revealing no toxicity of N-benzoyl-*L*-tyrosyl-*p*-aminobenzoic acid.

Effect of renal function on the pancreatic function test

In patients with known chronic renal insufficiency of varying extent, but with intact pancreas function, it was possible to show that the excretion of *p*-aminobenzoic acid is falsely low in the pancreatic function test. The results in controls and patients with renal disease show that the diagnostic value of the pancreatic function test decreases when serum creatinine values exceed 114.9 μmol/l. Since the anticipated increase of *p*-aminobenzoic acid excretion after administration of free as opposed to conjugated *p*-aminobenzoic acid occurred in only half of the patients with renal insufficiency, such a ratio cannot be utilized to make the test applicable for patients with renal disease. It appears that adequate renal function is a prerequisite to employment of this test.

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